

Atty Dkt. No.: CLON-035CIP  
USSN: 10/006,922

### **AMENDMENTS**

#### **In the Specification:**

**Please replace the title with the following rewritten title:**

**Nucleic Acids Encoding Novel Chromophores/Fluorophores and Method For Using The Same**

**Please replace the Abstract with the following rewritten Abstract:**

Nucleic acid compositions encoding novel chromo/fluoroproteins and mutants thereof, as well as the encoded proteins ~~encoded by the same~~, are provided. The subject proteins of interest are proteins that are colored and/or fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that they are either obtained from non-bioluminescent Cnidarian, e.g., Anthozoan, species or are obtained from non-Pennatulacean (sea pen) species. Specific proteins of interest include proteins obtained from the following specific Anthozoan species: Anemonia majano (NFP-1), Clavularia sp. (NFP-2), Zoanthus sp. (NFP-3 & NFP-4), Discosoma striata (NFP-5), Discosoma sp. "red"(NFP-6), Anemonia sulcata (NFP-7), Discosoma sp "green" (NFP-8), and Discosoma sp."magenta" (NFP-9). Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compositions find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compositions, are provided.

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**Please replace the paragraph on page 1, lines 5-23 with the following rewritten paragraph:**

This application is a continuation-in-part of Application Serial No. PCT/US00/28477 filed on October 13, 2000; which application is a continuation-in-part of the following applications: application serial no. 09/418,529 filed October 14, 1999, **now abandoned**; application serial no. 09/418,917 filed October 15, 1999; application serial no. 09/418,922 filed October 15, 1999; application serial no. 09/444,338 filed November 19, 1999; application serial no. 09/444,341 filed November 19, 1999; application serial no. 09/457,556 filed December 9, 1999; application serial no. 09/458,477 filed December 9, 1999; application serial no. 09/458,144 filed December 9, 1999; and application serial no. 09/457,898 filed December 9, 1999; all of which applications claim priority to application serial no. 09/210,330 filed December 11, 1998; as well as application serial no. 60/211,627 filed on June 14, 2000; application serial no. 60/211,687 filed on June 14, 2000; application serial no. 60/211,609 filed on June 14, 2000; application serial no. 60/211,626 filed on June 14, 2000; application serial no. 60/211,880 filed on June 14, 2000; application serial no. 60/211,607 filed on June 14, 2000; application serial no. 60/211,766 filed on June 14, 2000; application serial no. 60/211,888 filed on June 14, 2000; and application serial no. 60/212,070 filed on June 14, 2000; as well as International Application Serial No. PCT/US99/29405 filed December 10, 1999, which application claims priority to application serial no. 09/210,330 filed December 11, 1998; the disclosures of which application are incorporated in their entirety herein.

**Please replace the paragraph on page 9, lines 25-35 with the following rewritten paragraph:**

In these embodiments, the nucleic acid compositions are found in, derived from, or are mutants or homologues of, nucleic acids found in Anthozoan organisms from Sub-class Alcyonaria, often Order Stolonifera, and more often the Family Clavulariidae,

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where the organism is usually from the Genus *Clavularia*, and in certain embodiments, the organism is *Clavularia sp.*, where the specific wild type fluorescent protein of interest is cFP484 (i.e., NFP-2; RCFP-2). The wild type cDNA coding sequence for cFP484 is provided in ~~SEQ ID No: 03~~ **SEQ ID NO: 03**. In addition to nucleic acids encoding the wild type sequence and fragments thereof, also of interest are nucleic acids that encode homologues and mutants of the wild type protein. Specific mutants of interest include, but are not limited to:  $\Delta 19$  cFP484 and  $\Delta 38$  cFP484, where these specific mutants are further described in the experimental section, *infra*.

**Please replace the paragraph on page 10, lines 1-11 with the following rewritten paragraph:**

In these embodiments, the nucleic acid compositions are found in, derived from, or are mutants or homologues of, nucleic acids found in Anthozoan organisms from Sub-class Zoantharia, often Order Zoanthidea, more often Sub-order Brachycnemia, usually Family Zoanthidae, and more usually Genus *Zoanthus*, where in certain embodiments, the organism is *Zoanthus sp.*, where the specific wild type fluorescent protein of interest is zFP506 (i.e., NFP-3; RCFP-3). The wild type cDNA coding sequence for zFP506 is provided in ~~SEQ ID Nos: 05~~ **SEQ ID NO: 05**. In addition to nucleic acids encoding the wild type sequence and fragments thereof, also of interest are nucleic acids that encode homologues and mutants of the wild type protein. Specific mutants of interest include, but are not limited to: N66M; NFP-3NA (a non-aggregating mutant); yellow; yellow bright, etc., where these specific mutants are further described in the experimental section, *infra*.

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**Please replace the paragraph on page 42, lines 17-29 with the following rewritten paragraph:**

The Mut15-mdm2 fusion was generated by the following steps: first, mdm2 DNA was obtained by amplifying human Marathon cDNA library (Burke's Lymphoma) using primers:

ATGTGCAATACCAACATGTCTGTACC (~~SEQ ID No. 18~~ SEQ ID NO: 19) and CTAGGGGAAATAAGTTAGCAC (~~SEQ ID No. 20~~ SEQ ID NO: 20); secondly, the purified PCR product was then amplified with primers:

GGAATTCCAGCCATGGTGTGCAATACCAACATGTCTGTACC (~~SEQ ID No. 21~~ SEQ ID NO: 21) and TCCCCCGGGGGGAAATAAGTTAGCAC (~~SEQ ID No. 22~~ SEQ ID NO: 22)

in order to add Kozac sequence and restriction sites; thirdly, the purified PCR product from step 2 was digested with EcoR I and Sma I and inserted into EcoR I and SmaI of NFP1Mut15-N1 vector (this vector was generated using BamH I and Not I sites of the pEGFP-N1 backbone). The generated Mut15-mdm2 fusion was then expressed in HEK293 cells.

**Please replace the paragraph on page 50, line 37 to page 51, line 7 with the following rewritten paragraph:**

Non-humanized wild type coding region fragments from drFP583 and dmFP592 were amplified by PCR (22 cycles, 95°C, 15 sec., 68°C 1 min 20 sec.) using 1 ng of corresponding bacterial expression plasmids (pQE-30 derivatives with drFP583 and dmFP592 inserts, respectively) as templates. Oligonucleotides

A (ACATGG ATCCAGGTCTTCCAAGAATGTTATC, ~~SEQ ID No. 23~~ SEQ ID NO: 23),

B (TAGTACTCG AGCCAAGTTCAGCCTTA, ~~SEQ ID No. 24~~ SEQ ID NO: 24),

C (ACATGGATCCAG TTGTTC CAAGAATGTGAT, ~~SEQ ID No. 25~~ SEQ ID NO: 25),

and

D (TAGTACTCGAGGCCATTA CCGCTAATC, ~~SEQ ID No. 26~~ SEQ ID NO: 26)

were used as primers for amplifying these fragments in a concentration of 0.2 mM.

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**Please replace the paragraph on page 51, lines 20-38 with the following rewritten paragraph:**

The ligation mixture was then diluted by water ten-folds, and 1 ml of the dilution was preformed for PCR reaction (20 cycles, 95°C, 15 sec. 68°C 1min 20 sec) as template. Four oligonucleotides A, B, C, and D (~~SEQ ID Nos. 58-64~~ **SEQ ID NOS: 58-61**, respectively) were used simultaneously as primers for amplifying these fragments in a concentration of 0.1 mM each. After electrophoresis in an agarose gel (1.5%), the target fragment was purified by QIAquick Gel Purification Kit (QIAGEN) and digested with restriction endonucleases BamHI and XhoI (30-50 U each) simultaneously in BamHI restriction buffer (NEB) supplemented with BSA for 3h at 37°C. After purification, the resulting fragment was cloned in pQE-30 plasmid linearized by BamHI and Sall. Ligation reaction was performed in 1X T4 DNA ligation buffer and 400 NEB U of T4 DNA ligase with a total volume of 20 ml for overnight at 16°C. After transformation of *E.coli* cells by 1/5 of the ligation volume and incubation on LB/1% agar plates which were supplemented by 100 mg/ml Ampicilin and 0.1 mM IPTG at 37°C for overnight, the resulting *E.coli* colonies were screened visually under fluorescent microscope using rhodamine filter set. The brightest red colonies were picked up and placed in 200 ml LB medium with 100 mg/ml of Ampicilin. At OD<sub>600</sub>=0.6, the *E.coli* culture was induced by IPTG (final concentration was 1 mM) and the fermentation continued for overnight. Purification of recombinant protein containing N-terminus 6xhis tag was performed using TALON metal-affinity resin according to manufacturer's protocol.